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CRYOSURGERY

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■ **Abstract** Cryosurgery is a surgical technique that employs freezing to destroy undesirable tissue. Developed first in the middle of the nineteenth century it has recently incorporated new imaging technologies and is a fast growing minimally invasive surgical technique. A historical review of the field of cryosurgery is presented, showing how technological advances have affected the development of the field. This is followed by a more in-depth survey of two important topics in cryosurgery: (*a*) the biochemical and biophysical mechanisms of tissue destruction during cryosurgery and (*b*) monitoring and imaging techniques for cryosurgery.

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HISTORY OF CRYOSURGERY

Cryosurgery, sometimes referred to as cryotherapy or cryoablation, is a surgical technique in which freezing is used to destroy undesirable tissues. Although the prefix "cryo" (from the Greek word "kruos" for cold) usually refers to temperatures

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below 120 K (the definition adopted by the XIIIth Congress of the International Institute of Refrigeration), cryosurgery deals with temperatures below the freezing temperature of tissue, i.e. about 273 K. The history of cryosurgery is relatively short and is closely intertwined with developments in low temperature physics, engineering, and instrumentation that were made during the last century. A review of the history of the field will show that cryosurgery appears to advance in jumps triggered by immediately preceding technological advances.

Second Half of the Nineteenth Century

Toward the middle of the nineteenth century, physicists became interested in achieving and studying low temperatures. By mixing ice with various solutes, they were able to reach temperatures as low as 223 K with a mixture of ice and calcium chloride (1). Around 1845, Michael Faraday achieved a temperature of 163 K by mixing solid carbon dioxide and alcohol under vacuum. During the same period, James Arnott of Brighton, England, who is recognized as the first physician to use freezing for treatment of cancer, began applying these low temperatures in medicine. Most of his work focused on the use of cold in anesthesia (2). However, in several reports published between 1845 and 1851, he describes the use of a solution of crushed ice and sodium chloride to freeze advanced cancers in the breast and the uterine cavity (3). He used a water cushion cooled by the flow of a solution from a reservoir of brine. The cushion was applied to accessible tumors in the breast and the uterus. The advanced tumors, which were frozen to temperatures of about $-12°C$, developed a white, hard appearance upon freezing. After thawing, they became much less offensive, with no discharge or hemorrhage. Although the value of this procedure was recognized by Arnott's contemporaries and was incorporated in textbooks on the treatment of cancer (4), very few reports on the use of freezing for tissue destruction had been published by the end of the nineteenth century.

The second part of the nineteenth century witnessed several major discoveries in the field of cryogenics. In 1877, Cailletet of France and Pictet of Switzerland began developing adiabatic expansion systems for cooling gases. This led to the liquefaction of oxygen, air, and nitrogen. In 1892, Dewar of Great Britain designed the first vacuum flask, which facilitated storage and handling of liquefied gases. In 1895, Linde of Germany and Hampson of England began using throttle expansion (the Joule-Thomson effect) to produce continuously operating air liquefiers. At the end of the century, solid carbon dioxide, liquid air, and other gases were readily commercially available. The advances in the production of liquid cryogenic gases that occurred around the turn of the century triggered a resurgence of cryosurgery. This "rebirth" is commonly attributed to either Openchowski or White. In 1833, Openchowski reported using a low-temperature system for freezing portions of the cerebral cortex of dogs (5). However, in his study, freezing was used primarily as a means of inducing lesions in the brain rather than for therapeutic uses. In 1899, Campbell White, a physician from New York, reported the use of liquid air for

the treatment of diverse skin diseases (6). In his report White mentions that it was Professor Charles Tripler from New York who had the capability of making liquid air in large quantities. Apparently, for the previous two years, Tripler had urged the use of liquid air in therapeutics and supplied the liquid air to various physicians. Additional reports on the use of liquid air in therapeutics soon followed (7, 8). Several methods were developed for the application of liquid air to undesirable tissue. They include the use of a cotton swab saturated with liquid air, liquid air sprayed from a bottle, and liquid air–filled glass or brass containers that were rolled over the tissue. Liquid air was used for treatment of various diseases of the skin, such as warts, varicose leg ulcers, carbuncles, herpes zoster, epitheliomas, and erysipelas. Although liquid air appears to have been an efficient cryogen for treatment of undesirable tissue, it was not readily available to physicians and therefore fell into disuse (9). Apparently, there are no reports on the use of liquid air after about 1910 (10). Solid CO_2 was first used for therapeutics in 1907 by William Pusey, and soon became the most popular method of tissue freezing during the first half of the century (11). Solid CO_2 is readily available, as it is produced from expansion of compressed, liquefied CO_2 to atmospheric pressure. With the wide availability of solid CO_2, cryotherapy, as it was coined by Lortat-Jacobs & Solente in the first monograph on the field, became an established therapeutical technique in dermatology and gynecology (12).

First Half of the Twentieth Century

The field of cryosurgery experienced very few advances from the 1930s to the 1960s. Liquid oxygen became commercially available in the 1920s with the development of large new air separation facilities, such as the Linde Company air separation plants that use regenerators (1). Shortly after liquid oxygen became commercially available, it began to be used in the treatment of skin diseases in 1929 (13). However, because liquid oxygen is a fire hazard, it has never become a popular cryogen for cryosurgery. The development of chloro-fluorocarbon refrigerants led to the first closed-cycle refrigeration cryosurgery system in 1942 (14). Closed refrigeration cycles never became popular, probably because the temperatures they can achieve are much higher than those that can be achieved with the relatively less expensive use of solid CO_2. Starting from the early 1940s, Kapitsa in the Soviet Union and Collins in the United States began developing commercial techniques for large-scale liquefaction of hydrogen and helium, with liquid nitrogen as an abundant and low-cost by-product (1). Soon after liquid nitrogen became readily commercially available, Allington introduced it in clinical practice in 1950 (15). The liquid nitrogen was applied with a cotton swab, and soon became common in treatment of verrucae, keratoses, and various non-neoplastic lesions (10).

Prior to the 1960s, the devices used for cryosurgery were not efficient and were able to freeze only to a depth of several millimeters. Therefore, with a few exceptions, freezing was used primarily for the treatment of superficial layers of undesirable tissue, most often in the fields of dermatology and gynecology.

A notable exception is the pioneering work of Temple Fay, who in about 1939 treated patients with advanced carcinoma, glioblastoma, and Hodgkin's disease with local freezing (16). Fay employed freezing through irrigation of pads and implanted metal capsules in a technique reminiscent of Arnott's original method in the 1850s. Other scientists have also been using freezing as a means of destroying tissue deep in the body since the 1883 work of Openchowski. However, their use of the destructive effect of freezing was not for therapeutic purposes but rather as a means for studying the function of the destroyed tissues (17, 18). The year 1959 produced several scientific results that led to the emergence of "modern" cryosurgery. Several scientists reported devices for freezing of brain tissue in 1959. These devices employed cannulas through which refrigerants were circulated. Rowbotham and his colleagues used an alcohol mixture as a refrigerant (19), and Tytus & Ries used freon (20). Also in 1959, the Linde Company developed a new reflectory shield that, when incorporated in a vacuum insulation system, made it possible to achieve much greater insulation around a cryogenic container (1).

Modern Cryosurgery

Modern cryosurgery began through the collaborative work of a physician, Irving Cooper, and an engineer, Arnold Lee (21). They built a cryosurgical probe capable of freezing brain tissue, with good control over the site where the cryogenic lesion was produced. Their cryosurgery probe is essentially the prototype from which every subsequent cryosurgical probe using liquid nitrogen was built. The probe, made of three long concentric tubes, is supplied with liquid nitrogen from a pressurized source. The inner tube serves as a conduit for liquid nitrogen flow to the tip of the probe. The space between the inner tube and the middle tube serves as a path for the return of gaseous nitrogen from the tip of the probe. The space between the outer tube and the middle tube is vacuum insulated and has a radiative shield, which essentially allows the liquid nitrogen to be conducted without heat loss to the tip of the probe. The tip of the probe is a chamber into which the liquid nitrogen flows from the inner tube and from which the gaseous nitrogen returns through the space between the inner and the middle tube. Freezing takes place in the tissue around the chamber on the tip of the probe. The design of this probe allows controlled freezing of deep body tissue and control over the site at which freezing is induced. After the introduction of this cryosurgical probe by Cooper and Lee, the field of cryosurgery began to experience rapid growth, which lasted to the end of the decade. Gage recognized the important contribution of Cooper to the field of modern cryosurgery in a eulogy (22). Although originally designed for treatment of parkinsonism and other neural disorders, Cooper (23, 24) and others soon recognized the value of the cryosurgical probe for broad use in destroying undesirable tissue deep in the body.

Many new applications of cryosurgery were introduced between the years 1961 and 1970. It is impossible to list all the major contributions that were made to the

field during that period, and I will highlight only a few. Cahan and his collaborators applied cryosurgery to the uterus (25). Rand and his colleagues expanded the use of cryosurgery in neurology (26). Gonder and Soanes and their colleagues were the first to apply cryosurgery to the prostate (27). Marcove & Miller applied cryosurgery to orthopedics (28). Torre and Zacarian and their colleagues made advances in skin cryosurgery (29, 30). Gage investigated freezing in a broad range of tissues (10, 31, 32). Because the new cryosurgical devices required careful engineering design, several engineers such as Rinfret and Barron began publishing analyses of the cryosurgical devices during the 1960s (33, 34). A short-lived journal entitled *Journal of Cryosurgery* was published during that period. The advances made in cryosurgery during the 1960s are summarized in two comprehensive monographs (35, 36).

In cryosurgery the freezing probe or cryogen is applied to a particular tissue site, and the freezing domain propagates outwards from the site of application into the tissue. Therefore, the extent of tissue affected by the treatment is much greater than the tissue in contact with the cryogen or the probe. As such, cryosurgery is probably the first minimally invasive surgical technique. The cryosurgical probes developed in the 1960s allow for precise application of cryosurgical treatment deep in the body. This unique ability made cryosurgery very promising and resulted in the expansion of the method during this era. However, the minimally invasive nature of cryosurgery leads to difficulties with controlling the procedure. First, because the freezing propagates from a probe or a cryogen outward, the extent of the tissue affected by freezing cannot be determined visually by the surgeon, unlike the more conventional surgical resection techniques. Second, indiscriminant freezing itself does not necessarily destroy the tissue (see next section). Therefore, while the new cryosurgical probes could be applied at a precise location, their effect on the tissue treated by freezing was not precise. This lack of precision was soon recognized by physicians using cryosurgery and led to a reappraisal and eventual decrease in the use of the method during the 1970s. Cryosurgery lost its popularity even for surgical applications in which it had a proven beneficial effect. The incentive to use cryosurgery for treatment of parkinsonism disappeared when drug therapy using L-dopa became available. The use of cryosurgery for treatment of prostate cancer was replaced by more precise resection surgical methods. Laser techniques began to replace cryosurgery as a new technology for destruction of undesirable tissues. By the early 1980s, the field of cryosurgery had essentially reverted back to the original applications in which it was traditionally employed: dermatology and gynecology.

To apply cryosurgery precisely, it is imperative to know the following: (*a*) what the mechanisms of tissue destruction during cryosurgery are, and (*b*) how to evaluate the extent of tissue freezing and the thermal history in the frozen lesion. Advances in these areas have caused the recent resurgence in the field of cryosurgery. In the following sections I discuss advances in understanding the mechanisms of damage during cryosurgery and in methods for monitoring the process of freezing during cryosurgery and novel cryosurgical systems. While

trying to provide a balanced presentation, I found that the following sections focus primarily on work done in my laboratory. This is caused by space limitations coupled with what I perceive to be the need to focus on the fundamentals rather than a broad review of contributions. I apologize to the many deserving researchers whose work I omitted.

THE MECHANISMS OF TISSUE INJURY DURING CRYOSURGERY

Early on, Cooper recognized the need for precise information on the thermal parameters that destroy tissue. In 1964, he wrote that holding tissue at −20°C for one minute is sufficient to induce necrosis (37). During the 1960s, the basic thermal protocol for a cryosurgical procedure became rapid freezing, slow thawing, and repetition of freeze-thaw cycles (e.g. 37–39). It is obvious now that the thermal parameters used for freezing during cryosurgery were not precise and may have resulted in treatment failures. To control the outcome of cryosurgery it is important to understand the mechanisms of damage. Prior to a discussion of the mechanisms of damage it would be beneficial to provide a brief description of the thermal history that cells experience during cryosurgery.

In cryosurgery tissue is frozen with a cryosurgical probe that is brought in good thermal contact with the undesirable tissue. Usually, the probe is cooled through the internal circulation of a cooling fluid. The cooling fluid gradually extracts heat from the tissue, through the probe. Within several minutes after cooling begins, the temperature of the tissue in contact with the probe reaches the phase transition temperature and begins to freeze. As more heat is extracted, the temperature of the probe continues to drop and the freezing interface begins to propagate outward from the probe into the tissue. A variable temperature distribution in both the frozen and unfrozen regions of the tissue ensues. The freezing interface propagates outward until either the flow of the cooling fluid is stopped or until the heat that comes from the live tissue surrounding the frozen lesion becomes equal to the amount of heat that the cooling fluid in the cryosurgical probe can remove. At that time the frozen tissue has a temperature distribution that ranges from a low cryogenic temperature at the tissue surface in contact with the probe to the phase transformation temperature on the outer edge of the frozen lesion. The temperatures in the unfrozen tissue range from the phase transition temperature at the margin or the frozen lesion to the normal body temperature. In typical cryosurgical protocols, after freezing is completed the cooling system keeps the tissue frozen for a desired period of time, followed by heating and thawing. The primary mechanism for heating the frozen tissue is from the blood circulation and metabolism of the surrounding tissue. Sometimes the frozen tissue is also warmed from the probe surface by a warming fluid circulating through the cryosurgical probe. Depending on the physician's choice, the tissue is frozen again after complete or partial thawing. The cryosurgical procedure can be performed with several cryosurgical

probes to generate a particular shape of the frozen tissue, which corresponds to the shape of the undesirable tissue.

A typical cryosurgery procedure lasts between several minutes and an hour. Every cell of the tissue may experience a different thermal history. The cells near the cryosurgical probe surface will be cooled with a higher cooling rate and to lower temperatures than those farther away from the probe. The cells at different locations in the frozen lesion will be at different temperatures for various periods of time, as a function of their distance from the probe surface, the cooling fluid employed, the shape of the cryosurgical probes, the number of probes used, and the type of tissue frozen. The rest of this section discusses the relation between the particular thermal history that cells experience during cryosurgery and cell death. Cell damage during cooling and freezing occurs at several length scales: nanoscale (Angstrom), molecular; mesoscale (micron), cellular; and macroscale (millimeter), whole tissue. The time scales relevant to cryosurgery range between single minutes to tens of minutes. The thermal regime can be also divided into the temperature range from body temperature to the change of phase temperature of the body's physiological solution and the temperature range below the change of phase temperature. The damage during cryosurgery is of two types, acute, immediately during cryosurgery, and long term.

The Effect of Cooling

Most types of mammalian cells and tissues can withstand low, nonfreezing temperatures for short periods. The phenomena related to cooling occur primarily at the nanoscale, with typical consequences at the mesoscale. Cells are entities with a highly specific intracellular chemical content, separated from the nonspecific extracellular solution by the cell membrane. The cell membrane acts as a selective barrier between the intracellular and the extracellular milieu. The membrane selectively controls the transport of chemical species into and out of the cell. Therefore, the membrane must be mostly impermeable except at particular sites where it can control the mass transfer. The bilayer lipid structure of the cell membrane makes it impermeable. The mass transfer through the cell membrane is controlled through membrane proteins that span the membrane. Mammalian cells have become optimized to function at the temperature in which the organism lives. One aspect of cooling the cell to temperatures lower than their normal physiological temperature is the lipid phase transition process. The lipid membrane bilayer is in a fluid state during normal life temperatures. At lower temperatures and lower thermodynamic free energy the lipids undergo phase transition into a gel phase or into other three-dimensional structures with lower free energy. During the process membrane proteins become segregated and defects form between the proteins and the membrane bilayer. This phase transition process makes the cell membrane more permeable and allows (usually) ions to enter the cell in an uncontrolled way. Detailed reviews of the effect of temperature on the cell membrane can be found in several publications (40–42).

Normally the membrane proteins control the intracellular composition by selectively introducing and removing ionic species from the cell interior. However, life processes are temperature-dependent chemical reactions. Lowering the temperature also reduces the efficiency of the membrane proteins and their ability to control the intracellular content. Therefore, during cooling, the intracellular composition and in particular the intracellular ionic content begins to change as undesirable ions enter a cell by diffusion and are not removed. The damage is cumulative, a function of time, and is particularly expressed when the cells are returned to their normal physiological temperature. Additional mechanisms of damage relate to the cytoskeleton. The cytoskeleton structure depends on chemical bonds between membrane proteins and the cell scaffold. Lowering the temperature weakens these bonds and makes them particularly vulnerable to mechanical damage. A third mechanism of damage relates to the denaturation of proteins as a function of both temperature and change in the intracellular ionic content. Most cells and tissues can withstand brief cooling to above freezing temperatures, in the time scale typical of a cryosurgical procedure and under the cooling circumstances typical of cryosurgery. Therefore, it is not anticipated that tissues in areas around the frozen region would be severely damaged by the cooling they experience during cryosurgery. Major exceptions are cells that are highly sensitive to their ionic content, such as platelets. Cooling platelets to temperatures lower than their lipid phase transition temperature allows calcium influx, which appears to trigger platelet activation. This could lead to a cascade of events that would end in platelet aggregation and the eventual obstruction of blood vessels in the cooled region around the frozen lesions. Other cells whose function is strongly dependent on their ionic content are muscle cells, in particular in the heart and around arteries. These may be also damaged in the cooled region beyond the frozen lesion.

The Effect of Freezing

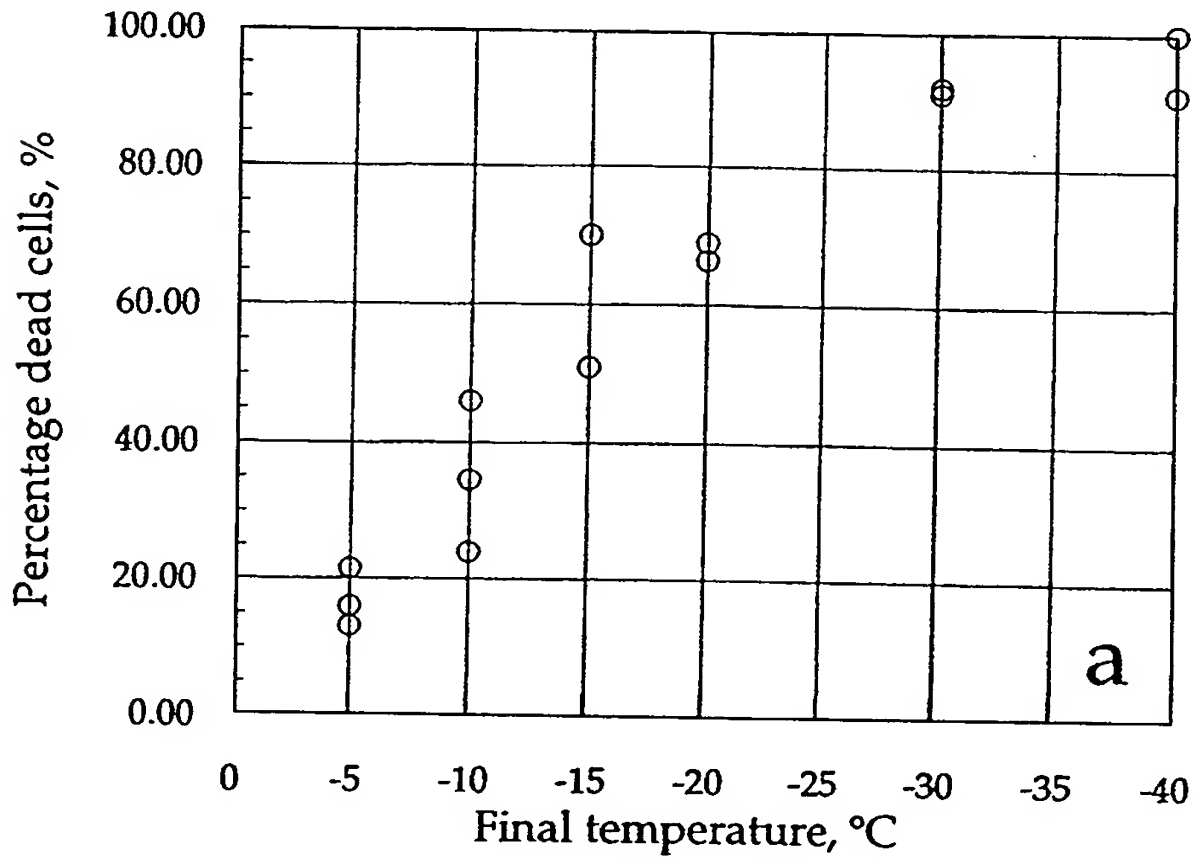
Freezing of biological tissues has been studied extensively. However, most of these studies were done in the field of cryopreservation, i.e. in relation to attempts to preserve cells and tissues in a frozen state for transplantation. The results of those studies are only indirectly relevant to cryosurgery and will not be discussed here. The thermal processes during freezing for preservation (cryopreservation) are different from the thermal processes during cryosurgery. In cryopreservation cells and tissues are frozen in vitro; they are usually frozen with uniform conditions to very low cryogenic temperatures and are kept in a frozen state for long periods of time. Most important, they are frozen in the presence of chemical additives that improve survival. In contrast, in cryosurgery the tissue is frozen in vivo; it experiences a large variation in cooling and warming conditions, and in a frozen state it experiences a wide range of temperature, from the phase transition temperature on the outer edge of the frozen lesion to cryogenic temperatures near the probe. In the field of cryopreservation it is traditional to think that the survival of frozen cells depends on the cooling rate (i.e. temperature change as a function of time)

during freezing (43). The relation between survival and cooling rates is traditionally depicted as an inverse U-shaped curve with optimal survival at a certain rate and decrease in viability at cooling rates above and below the optimal rate. It is important to realize that the inverse U-shaped curve is obtained in cryopreservation experiments in which the cells are frozen to cryogenic temperatures.

More relevant to understanding the mechanism of damage during cryosurgery are experimental results of the type depicted in Figures 1, 2 and 3 (44). In these experiments ND-1 prostate cancer cells were frozen with specified cooling rates to different subzero temperatures. The results are typical of other cells as well. The figures show that for cooling rates of 1°C/min and 5°C/min there is a gradual, almost linear increase in cell death to temperatures of about −40°C. For higher cooling rates of about 25°C/min there is a sudden steplike increase in cell death at a temperature of about −10°C. These results can be explained by the traditional explanation for the "inverse U shaped" survival curve proposed by Mazur (43).

Figure 4 is used to illustrate this explanation. Figure 4 was obtained through a directional solidification cryomicroscope and shows the process of freezing of cells (red blood) in suspension (45). The temperature distribution in these figures is one-dimensional linear, from below freezing at the left to above freezing at the right. The process of freezing in aqueous solutions is affected by the fact that ice cannot contain any solutes. Therefore, when an aqueous solution freezes, the solutes are accumulated in front of the change of phase interface. Although this phenomenon occurs on the microscale and is not visible, Figures 4*a* illustrates a similar macroscopic situation. The figure shows that at the beginning of the freezing process cells accumulate on the change of phase interface, which has the appearance of a vertical line. The increased solute concentration on the change of phase interface has the effect of colligatively lowering the temperature of the change of phase interface. Because thermal diffusion is much faster than mass diffusion the increased concentration and related change in phase transformation temperature leads to a phenomenon known as constitutional supercooling and the so-called Mullins-Sekerka interface instability. This phenomenon is discussed in detail in many material science texts, (e.g. 46). It causes the planar freezing interface to become unstable and take the fingerlike shape shown in Figures 4*a–c*. In this configuration the concentration of the solution at the tip of the fingerlike ice crystal structure is very close to the bulk solution concentration, and the rejected solutes become accumulated between the fingerlike ice crystal structures. The figure shows that the cells in the freezing solution are unfrozen and find themselves in the high concentration solute channels between the ice crystals. This is the hallmark of the process of freezing in biological materials. Although referred to as freezing of tissue or cells, in fact during most of the freezing processes the freezing begins in the extracellular milieu, and the interior of the cell is unfrozen.

Figure 5 illustrates a sequence of events that cells experience in the hypertonic solutions, between ice crystals (45). The figure shows that at lower temperatures, as the extracellular concentration increases, cells shrink. This shrinkage is caused by the fact that the unfrozen cells are supercooled relative to the extracellular solution,



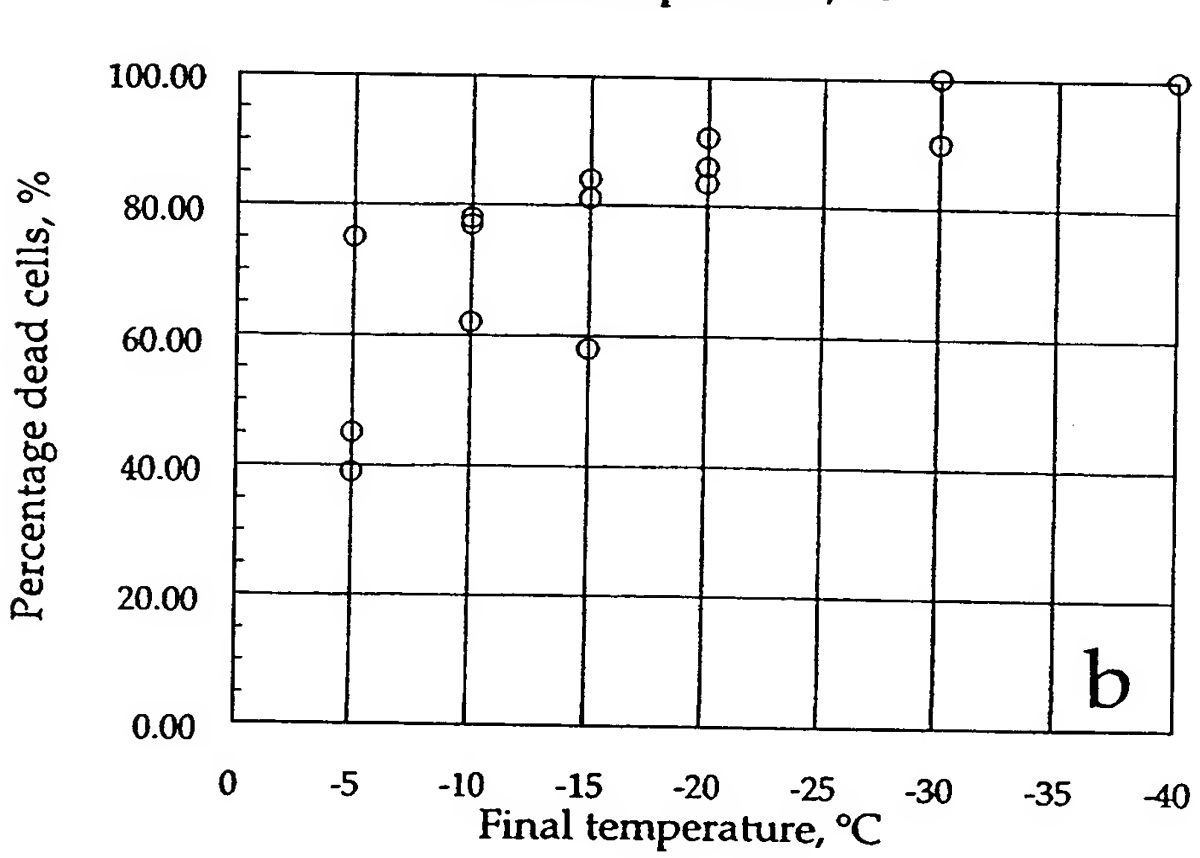


Figure 1 Percentage of prostate cancer cells, ND-1, destroyed by freezing with a cooling rate of 1°C/min as a function of the temperature to which they were frozen. (*a*) Single freeze/thaw cycle; (*b*) double freeze/thaw cycle.

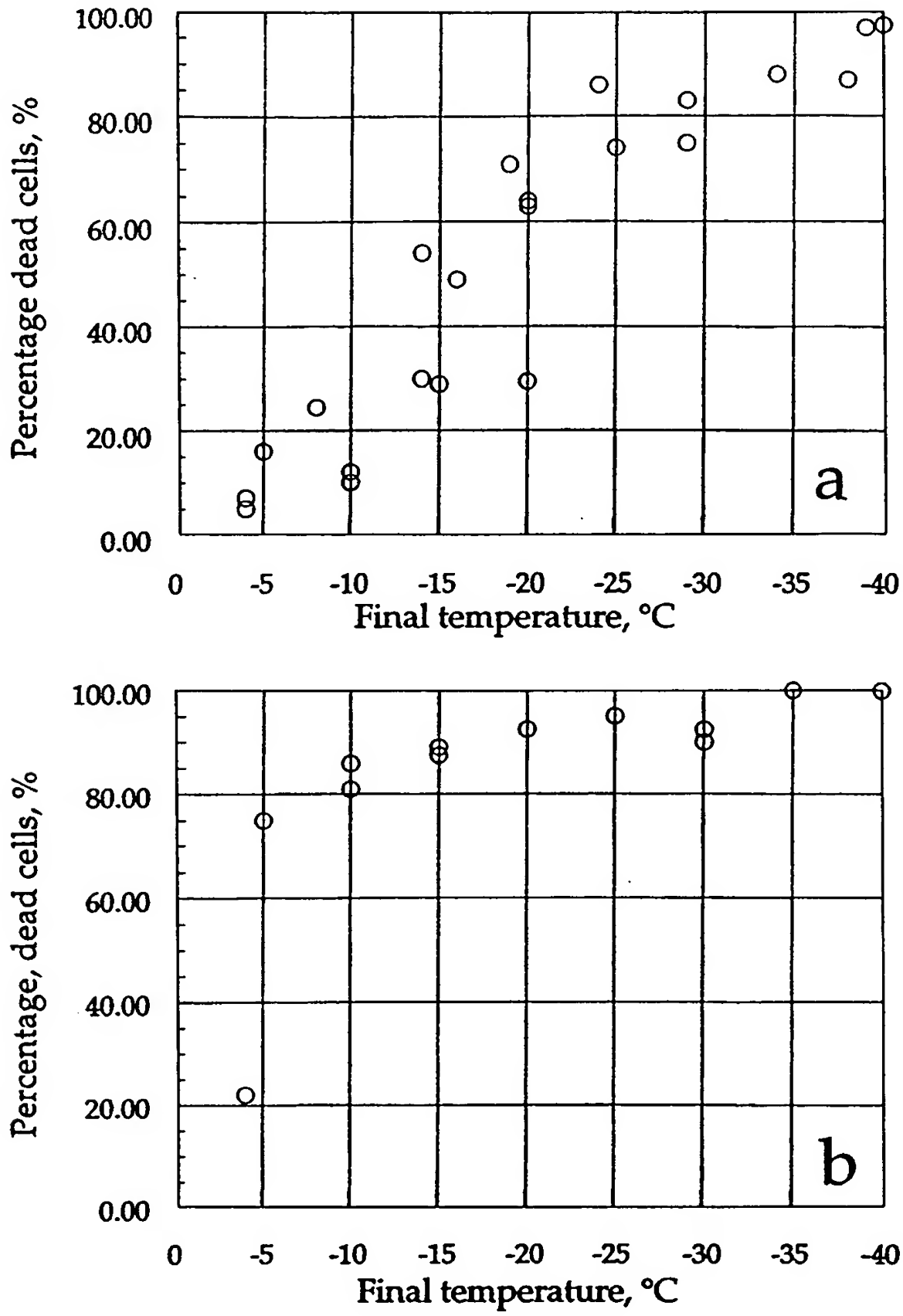
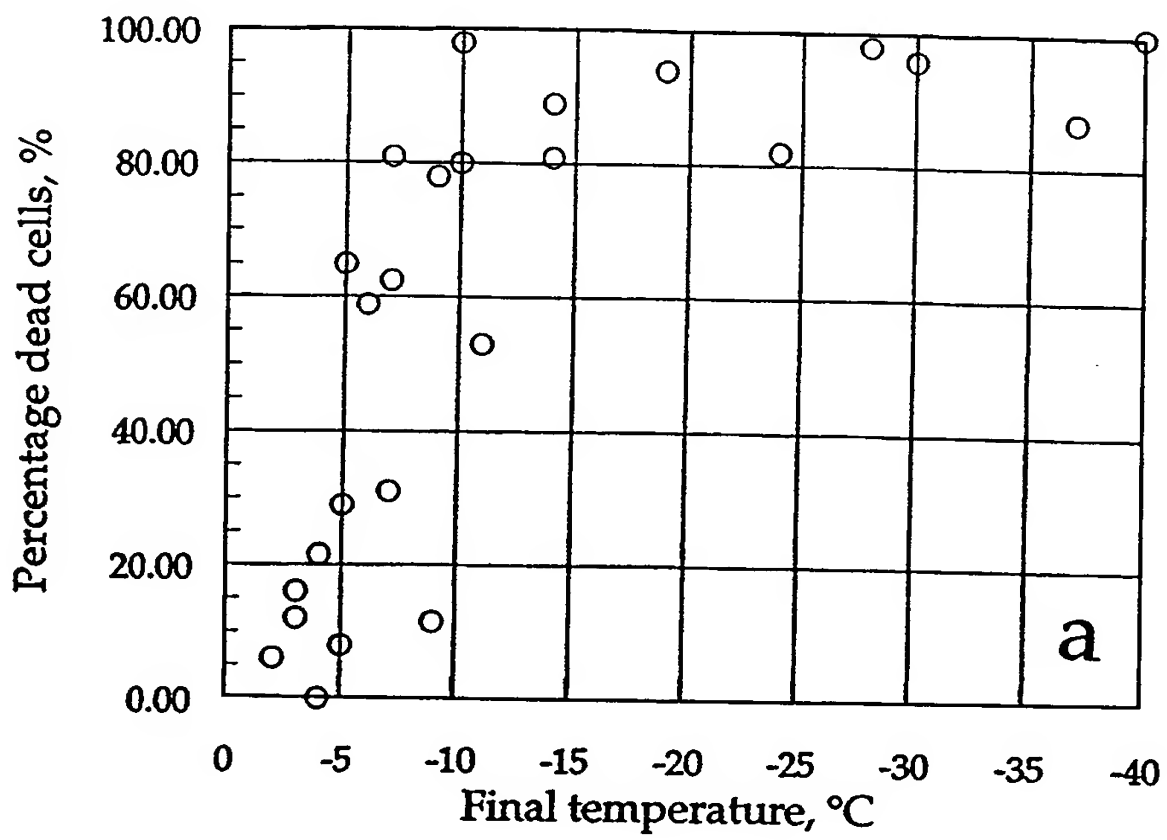


Figure 2 Percentage of prostate cancer cells, ND-1, destroyed by freezing with a cooling rate of 5°C/min as a function of the temperature to which they were frozen. (*a*) Single freeze/thaw cycle; (*b*) double freeze/thaw cycle.



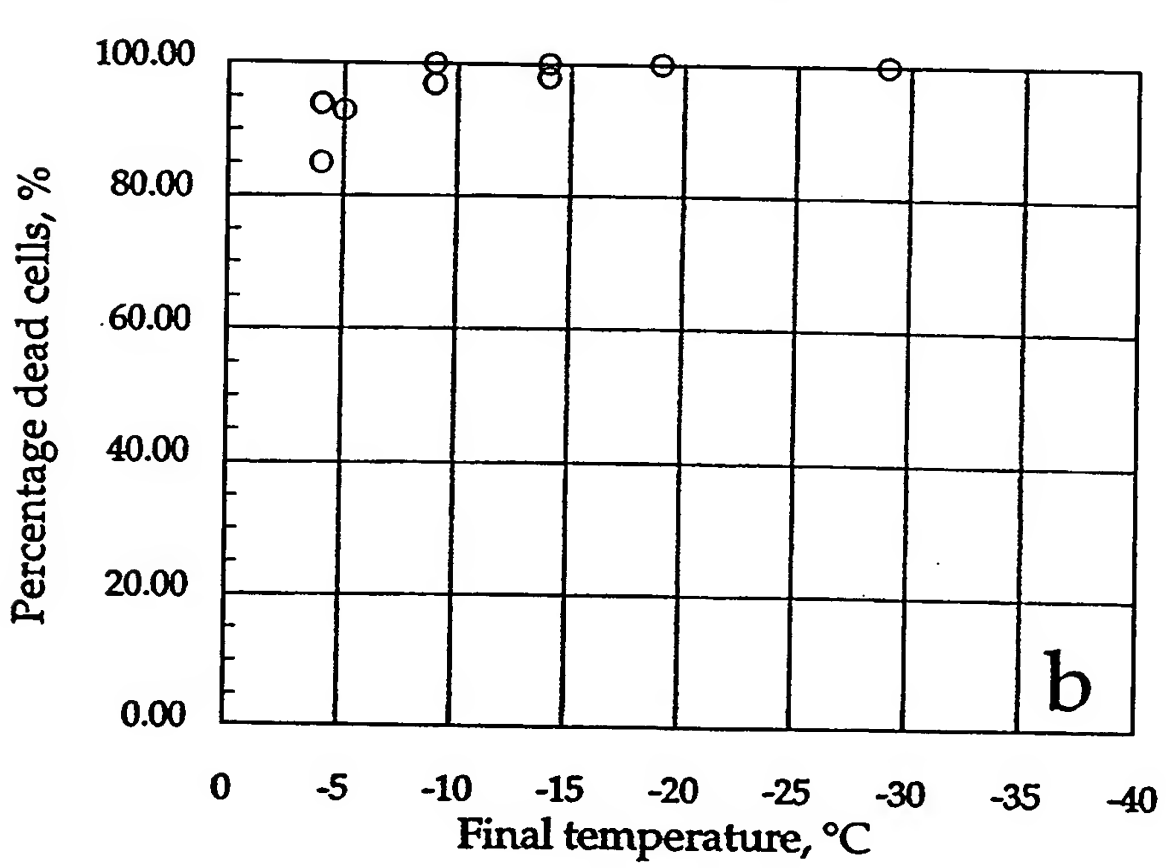


Figure 3 Percentage of prostate cancer cells, ND-1, destroyed by freezing with a cooling rate of 25°C/min as a function of the temperature to which they were frozen. (*a*) Single freeze/thaw cycle; (*b*) double freeze/thaw cycle.

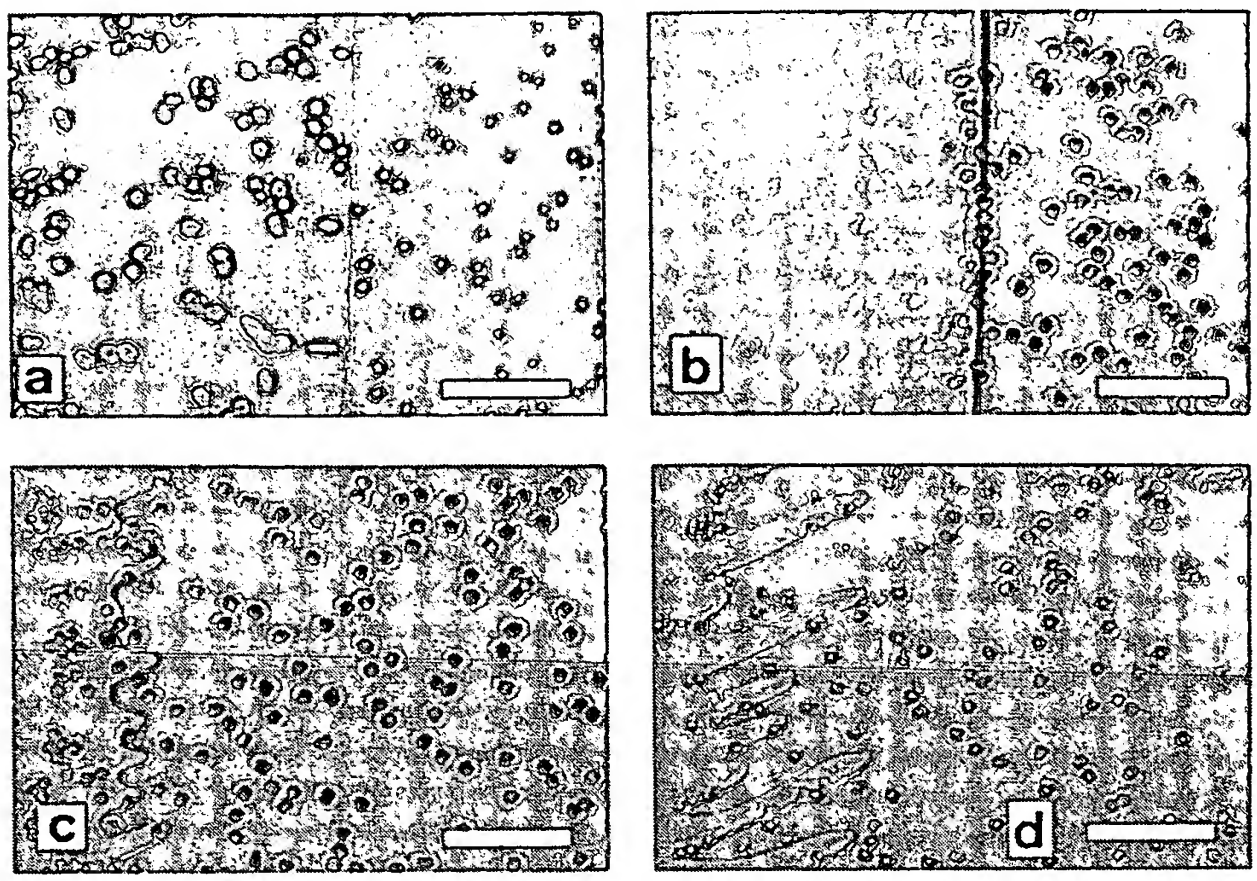


Figure 4 A sequence of photomicrographs showing the freezing of a suspension of red blood cells (*round circles*) in a physiological saline solution. The freezing interface is the *vertical line* in the middle of the figures. Figure 4*a* is the onset of freezing, and the subsequent figures show images taken during progressively longer times. The freezing interface propagates from left to right, with left-hand side frozen and right unfrozen. Scale bar 50 micron. (45).

which is in thermodynamic equilibrium with the ice. To equilibrate the difference in chemical potential between the extracellular and intracellular solutions, water will leave the cell through the cell membrane, which is readily permeable to water. This causes an increase in the intracellular solute concentration, with a decrease in temperature. It was originally proposed by Lovelock, (47) and later incorporated in Mazur's comprehensive theory (43), that increased hypertonic extracellular solutions damage the cells. The mechanisms are not entirely clear

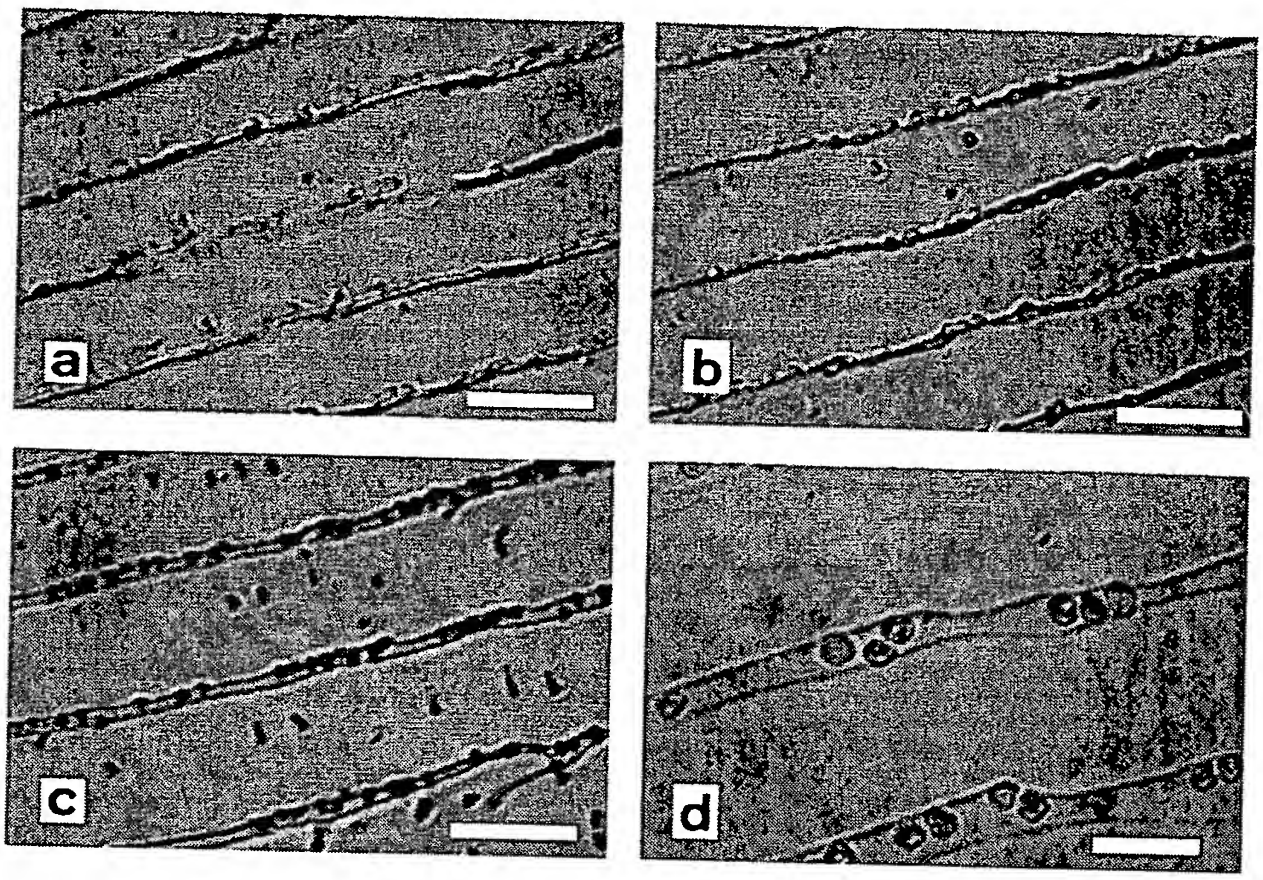


Figure 5 Red blood cells in the unfrozen hypertonic solution between ice crystals. Figures 5*a*,*b*,*c* were obtained in freezing experiments with cooling rates of 19.6°C/min and 5*d* in freezing experiments with cooling rates of 8.94°C/min. Images were taken as a function of distance from the outer tip of the ice crystal. (*a*) 0.75 mm, (*b*) 0.5 mm (*c*) 0.25 mm (scale bar 50 micron), and (*d*) 0.25 mm (scale bar 20 micron) (45).

and they could relate to chemical damage or osmolality-induced changes in the cell structure. Figures 1 and 2 show that indeed when cells are frozen to different subzero temperatures the percentage of damaged cells increases gradually. This is consistent with the hypertonic extracellular solution mechanism of damage, as the hypertonic extracellular concentration also increases gradually with a decrease in temperature.

There are several additional phenomena worth mentioning in relation to the hypertonic mode of damage. While little attention has been paid to this observation, the original experiments of Lovelock showed that the damage produced by exposure to hypertonic solutions was rapid and, within the limits studied, seemed to be independent of temperature and time of exposure. Exposing prostate cancer cells to different extracellular hypertonic solutions and examining the survival of the cells as a function of time produced results such as those illustrated in Figure 6 (48). Figure 6 is consistent with Lovelock's original results. The figure shows that while cell death increases with extracellular concentration, time affects survival only during the first few minutes of exposure, after which a plateau is reached and the percentage of death cells remains constant. A definite explanation for a

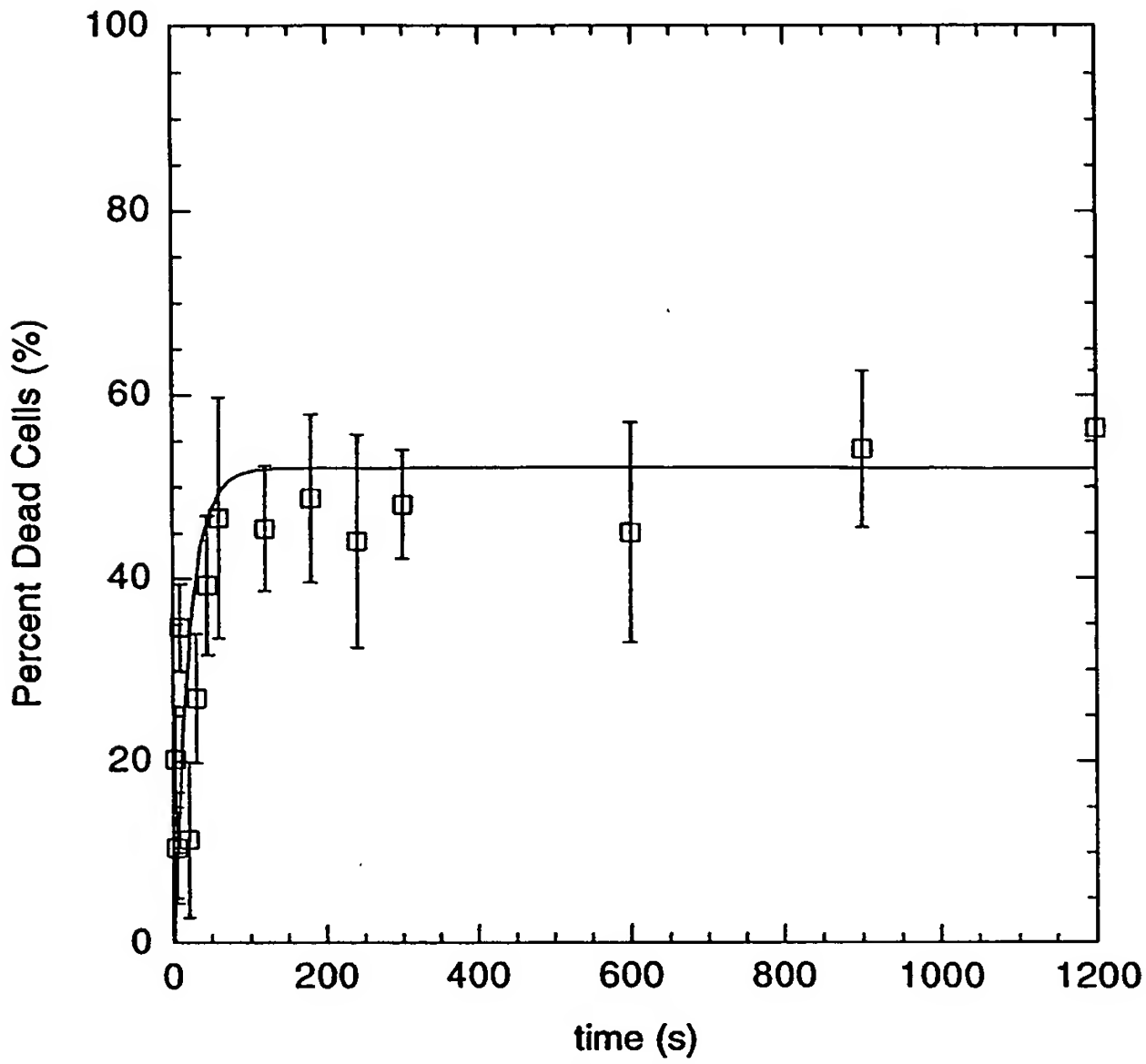


Figure 6 Percentage of prostate cancer cells destroyed by exposure to a 2.0 molar NaCl solution versus the time of exposure at 6°C (111).

mechanism of damage during hypertonic exposure has eluded researchers. This is because while the mesoscale processes that occur during exposure of cells to hypertonic solutions (cell shrinkage as water leaves through the cell membrane) have been observed and are understood, the nanoscale processes are not. However, in cryosurgery these mechanisms of damage are more important than in cryopreservation because many cells in the frozen region will remain throughout the procedure in the region dominated by hyperosmotic phenomena where the solution is partially frozen and the cells are not. The significance of these results is that if the only mechanism of damage is hyperosmotic, then keeping the cells in a partially frozen state will not significantly increase cell destruction. Also, in the partially frozen region cell destruction will not be complete, as indicated by Figures 1 and 2.

There are, however, additional mechanisms of damage in the area of temperatures and cooling rates associated with hypertonic solution damage. These modes of damage were originally observed by Nei (49) and later by Mazur (50) in his so-called unfrozen fraction hypothesis. Experiments have shown that the percentage of death cells after freezing is larger than the percentage of death cells after exposure to a similar extracellular hypertonic solution. This suggests that mechanical interaction between ice and cells may contribute to cell death. This is a reasonable assumption, since ice rejects cells in the space between ice crystals, as shown in Figure 4*d* and Figure 5. This may generate a mechanical force on the cells, whose cellular cytoskeleton is weakened by cold, and destroy them (51). Another possible mode of damage is the contact and interaction between ice and the lipid bilayer, which by itself may be damaging (42).

Figure 3 shows that for a cooling rate of 25°C/min, at a temperature of about −10°C there is a sudden decrease in cell destruction. Experiments have shown that this sudden increase in cell destruction corresponds to sudden formation of intracellular ice. Formation of intracellular ice has been also proposed to be responsible for the decrease in cell survival at above optimal cooling rates in cryopreservation experiments (43). The condition under which intracellular ice forms was investigated in several studies, starting with the work of Diller (52). It is thought that intracellular ice forms because the water transport through the cell membrane is a rate-dependent process. When cells are cooled too rapidly to equilibrate in concentration with the extracellular solution, the intracellular solution becomes increasingly thermodynamically supercooled and unstable. The probability for intracellular ice formation increases with supercooling. It is not clear if the nucleation sites for intracellular ice formation are intracellular, extracellular or on the membrane (53). However, whatever the cause of intracellular ice may be, it appears that it is almost always lethal to the cell. It is again unclear if the intracellular ice per se is lethal or if the processes that led to the formation of the intracellular ice, such as damage to the cell membrane, are lethal. As with the hyperosmotic solution mechanism of damage, with intracellular damage the mesoscale phenomena are known, whereas the nanoscale are not. In cryosurgery the mechanism of rapid cooling and intracellular ice formation usually occurs in the frozen lesion near the cryosurgical probe. It is thought that near the cryosurgical probe the cells are completely destroyed.

In cryosurgery the freezing cells are in tissue, which has a different configuration from a cellular suspension. In tissue, cells are in an organized structure and the volume of the extracellular space is usually smaller than that around cells in a suspension. It is natural to question whether the process of freezing and the mechanism of damage experienced by cells frozen in a suspension are similar to those in tissue. Whereas experiments with tissue are more difficult than those with cells, the few experimental results that exist show that the process of freezing of cells in tissue and in suspension is roughly similar. Experiments in which the different types of tissue were frozen with controlled thermal conditions and then viewed with electron microscopy or freeze-substitution show that in tissue ice usually

forms first in the extracellular space. Ice appears to form usually in the vasculature and propagate in the general direction of temperature gradients, but in and along blood vessels (54). In addition, it was found that in the prostate ice forms in the ducts (55), in the breast in the connective tissue (56), and in the kidney in the ducts (57). The cells in the various tissues appear to also experience cellular dehydration and intracellular ice formation. Figure 7 illustrates a typical appearance of slowly frozen liver tissue as seen with electron microscopy. The figure shows dehydrated hepatocytes surrounding expanded sinusoids. A mathematical analysis of the process of freezing in the liver compared the process of freezing of hepatocytes in the liver and in a cellular suspension (58). The results demonstrate that in both cases hepatocytes experience a similar dehydration process and a similar probability for intracellular ice formation. Therefore, cells in tissue will probably experience both qualitatively and quantitatively similar mechanisms of hypertonic solution damage and intracellular ice formation damage like cells frozen in cellular suspensions. However, the analysis suggests that in tissue the dehydration of cells will most likely result in a disruption of the vasculature and of the connective tissues. The consequence of this mode of damage to cryosurgery will be discussed later.

Thawing and Warming

Thawing and warming have been studied much less than freezing. However, they can also induce cellular damage. During warming, in a frozen state, ice has a tendency to recrystalize at high subzero temperatures, to minimize the Gibbs free energy (59). Recrystalization will cause further disruption of the extracellular space and may disrupt the macroscopic structure of the tissue (60). During thawing, as ice melts, the extracellular solution can be briefly and locally hypotonic, causing water to enter some cells and expand them and rupture the membrane. When the thawing is rapid some cells may remain hypertonic at body temperature, which could induce metabolic disruption and additional damage (61).

Thermal Parameters Specific to Cryosurgery

It is common in cryosurgery to employ double freeze-thaw cycles. Figures 1 to 3 illustrate the effect of a double freeze-thaw cycle on cells. Comparison with a single freeze-thaw cycle shows that the second freeze-thaw cycle will increase damage. Double and even triple freeze-thaw cycles are now commonly used in cryosurgery. The mechanisms of damage during multiple cycles are most likely related to cell membrane damage during the hypertonic variations that the cells experience upon freezing and thawing and with temperature variation.

Freezing and thawing can also induce another mode of macroscopic damage related to the thermal stresses that form in the frozen tissue as a function of the temperature variation in the ice. These stresses cause fractures that have been shown to occur experimentally and were studied analytically (62, 63). During cryosurgery, these fractures are usually to be avoided, as they cause uncontrollable damage to the tissue and bleeding (G Onik, personal communication).

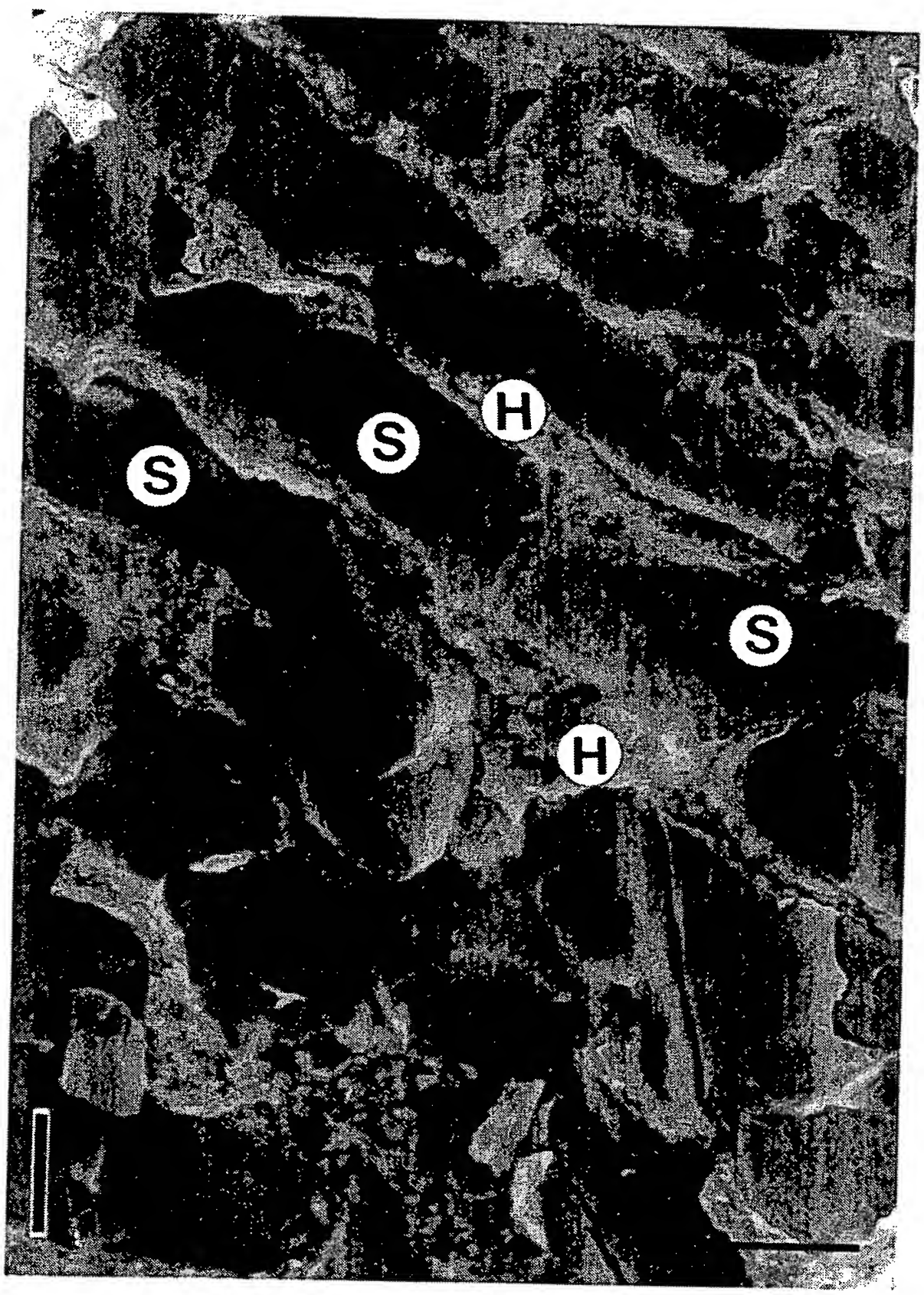


Figure 7 Typical appearance of slowly frozen liver with large ice crystals in the sinusoids (S) and dehydrated hepatocytes (H). Scale bar 20 micron.

Damage to the vascular system is probably one of the most important macroscopic mechanisms of tissue damage in cryosurgery. During cryosurgery the frozen region is obviously occluded from the blood circulation. Experiments show that immediately after thawing there is edema on the outer margin of the previously frozen lesion (64). Shortly thereafter the endothelial cells in the previously frozen region appear damaged, probably by the mechanism of blood vessel expansion during freezing, discussed earlier (65). Within a period of several hours after thawing, the endothelial cells become detached, with increased permeability of the capillary wall, platelet aggregation, and blood flow stagnation. Many small blood vessels are completely occluded within a few hours after cryosurgery. The loss of blood flow will ultimately result in ischemia and tissue death. It is thought that this mechanism of tissue destruction explains why cells appear to have succumbed to cryosurgery even in those areas in which the freezing parameters would normally not cause cell death. Cryosurgery is probably the first surgical technique that has used angeonesys to treat cancer.

Although most of the studies on the process of cell death during freezing have employed viability tests that evaluated survival of cells immediately after freezing and thawing, it appears that some cooling and freezing conditions may produce less lethal modes of damage, which eventually result in gene-regulated cell death (apoptosis). Apoptosis can be triggered by a variety of conditions present during cryosurgery, such as hyperosmolality. Apoptosis will take place after cryosurgery and can produce further cell death (66).

In addition to the verified mechanisms of tissue damage during cryosurgery there is anecdotal evidence that cryosurgery may result in a beneficial systemic immunological response (67). There is no doubt that a normal immune response exists in response to the tissue injury that freezing produces (68). However, the usefulness of this immune response in treating metastatic tumors is not certain.

Recently a new concept was developed that has the potential for increasing the destructive effect of freezing. It has been observed that a family of proteins known as antifreeze proteins has the ability to modify the structure of ice crystals. These proteins, found in a large number of cold tolerant animals and plants, noncolligatively inhibit the freezing temperature of solutions. However, when the solutions eventually freeze in the presence of these antifreeze proteins, they modify the structure of ice crystals (69). At certain concentrations these ice crystals can become needlelike and lethal to cells (70–72). In cryosurgery experiments, in which the antifreeze proteins were introduced in tissue prior to the procedure, it was found that the cells were destroyed by freezing throughout the tissue regardless of the thermal history employed during freezing (73–75). The mechanism of damage appears to be mechanical and related to the interaction between the ice crystals and the cells. It appears that the antifreeze proteins induce intracellular ice formation at high subzero temperatures, regardless of the thermal history during freezing. Obviously the use of antifreeze proteins as a chemical adjuvant to cryosurgery may become important. The destruction of frozen tissue

may potentially become independent of the thermal history that the cells have experienced during freezing.

Summary of Tissue Damage

The thermal history during cryosurgery is complex and so is the mechanism of damage. Cooling rates vary throughout the frozen lesion from uncontrollable high near the probe surface to low near the outer edge of the frozen lesion. Temperatures range from cryogenic near the probe to body temperature. This complex thermal history, combined with the complex mechanism of damage during freezing, makes it difficult to predict the outcome of a cryosurgery protocol and the relation between the extent of freezing and the extent of tissue damage. In view of the complexity of the process it is almost surprising that cryosurgery is producing good clinical results. Several standard procedures are now used in cryosurgery, loosely based on the fundamental studies described in this section. Because there appears to be no control over the cooling rates throughout the frozen tissue, temperature is taken to be the most common indicator for thermal damage. The lethal temperature is taken by different physicians to be between $-20°C$ and $-50°C$. In treating cancer they try to freeze beyond the margin of the tumor in such a way that the highest temperature the frozen tumor will experience is the limit they set. After freezing, it is common to hold the tissue in a frozen state for a while, then either to completely thaw the tissue or to thaw only the outer edge of the frozen lesion and repeat the freeze-thaw cycle once or even twice. At this stage much more research is needed to develop the ability to predict the relation between thermal history and tissue destruction during cryosurgery and to facilitate precisely controlled cryosurgery.

MONITORING CRYOSURGERY

To perform a cryosurgical procedure successfully, it is important to precisely monitor and evaluate the extent of freezing. Failure to do so accurately can lead to either insufficient or excessive freezing, and consequently, to recurrence of malignancies treated by cryosurgery or to destruction of healthy tissues.

Mathematical Models

Soon after the development of the first modern cryosurgical probes in the late 1960s attempts to develop mathematical models to predict the extent of tissue freezing during cryosurgery began. Trezek and Cooper were the first to develop mathematical models to describe and predict the extent of the frozen region during cryosurgery and the thermal history during the procedure (76, 77). Cooper and Trezek actually prepared a detailed atlas to be used in connection with brain cryosurgery (36). Their models employed traditional mathematical techniques developed earlier for engineering applications in the areas of welding and casting.

The major difference between the Cooper and Trezek models and the more conventional engineering heat transfer models was the use of the bio-heat equation in the unfrozen region. Comini & del Guidice began using the finite element technique to predict the extent of freezing in more realistic geometries soon after (78). Rubinsky & Shitzer were the first to try to optimize cryosurgery mathematically. They suggested the use of inverse mathematical techniques for designing optimal cryosurgical protocols. Their model used experimental biophysical data on the thermal parameters required for tissue destruction and combined this data with solutions to the inverse heat transfer equation (79, 80). Subsequently, Keanini & Rubinsky developed cryosurgery optimization protocols using the simplex optimization method (81). Rabin & Shitzer developed additional techniques for solving inverse problems in cryosurgery in the mid 1990s (82). They also developed additional mathematical techniques for predicting the extent of freezing during cryosurgery (83). There is no doubt that as the field of cryosurgery matures, mathematical models and optimization techniques will gain prominence. However, at this stage the medical profession has not yet embraced the use of mathematical techniques in cryosurgery.

Local Monitoring of Cryosurgery

One method to monitor the process of freezing during cryosurgery is with local measurement techniques. Cryosurgery is monitored locally, either through thermometry (e.g. 84–86) or through impedancemetry (87–90). Thermometry is based on direct measurements of temperature at discrete points in tissue with thermocouples or thermistors placed inside or around the undesirable tissue that is being frozen. William Cahan had already stressed the importance of temperature monitoring during cryosurgery in the early 1960s (36). At the end of the 1970s, the use of thermometry during cryosurgery had become routine, (e.g. 84).

During the late 1970s, several researchers, the first of whom were Le Pivert and his colleagues, suggested the use of local electrical impedance measurements to monitor cryosurgery (87). Le Pivert et al have shown that as tissue, which is essentially a solution of electrolytes, freezes, its ability to conduct electrical currents decreases and its impedance value increases from several kilo-Ohms in live tissue to several mega-Ohms in frozen tissue. Impedancemetry employs electrode needles placed locally inside or around the undesirable tissue that is being frozen and detects freezing-induced changes in local impedance. Local thermometry was compared to local impedancemetry in several studies, with conflicting results. Some have concluded that impedancemetry is preferable for monitoring cryosurgery (e.g. 90) and others that thermometry is preferable (e.g. 85). While local monitoring techniques are valuable and have contributed to cryosurgery, they have several drawbacks. First, the procedure is invasive and requires the insertion of either thermocouples or electrode needles into the tissue. Second, the information produced by local monitoring is restricted to the measured site. This means that either insufficient or excessive freezing can still occur elsewhere in

the frozen lesion. However, during cryosurgery, it is important to precisely monitor and evaluate the extent of freezing. Failure to evaluate correctly the extent of freezing can lead to either insufficient or excessive freezing and consequently, to malignancy recurrence or to destruction of healthy tissue. This problem was not resolved with local monitoring of cryosurgery. As indicated earlier, this lack of freezing control is what ultimately led to the decline in the use of cryosurgery in the 1970s.

Imaging Monitored Cryosurgery

Cryosurgery experienced a revival in the mid 1980s which can be attributed to the work of Onik, a radiologist, and Rubinsky, an engineer, who began the field of imaging-monitored cryosurgery. As with other advances in cryosurgery, the emergence of imaging-monitored cryosurgery is also closely related to preceding technological advances. The ability to image the whole human body is probably one of the most important advances in twentieth century medicine. X-rays were the first medical imaging technology of the twentieth century. Advances in computers and microprocessors in the 1970s were soon coupled with other technologies to develop X-ray computed tomography, magnetic resonance imaging, and ultrasound (91).

The first imaging technique used in clinical cryosurgery was ultrasound. In debating the advantages of computer tomography and ultrasound, the group of Onik and Rubinsky chose to begin developing the field of imaging-monitored cryosurgery with ultrasound because it was easier to use and less expensive (92).

Several techniques are available for acoustic imaging of the body. In the more common pulse-echo technique a short pulse of electrical energy is converted into a burst of acoustic energy with a piezoelectric transducer. The pressure wave that is produced propagates through the body. When the pressure pulse encounters the boundary between regions with different acoustic impedance, part of the wave is reflected back to the transducer, where it is converted back to an electrical impulse. The piezoelectric transducer functions as both an emitter and a detector. In whole-body imaging it is assumed that the velocity of the acoustic wave is approximately 1450 m/sec. When pressure waves return to the piezoelectric element, the measured time of flight of these waves can be combined with knowledge of the tissue wave speed to determine the location of the acoustic impedance discontinuity. Two-dimensional images of acoustic discontinuities in tissue can be produced using multiple piezoelectric elements and computer analysis of the data. The accuracy of ultrasound images is limited by the assumption of the wave velocity in tissue. Freezing interfaces can be conveniently monitored with conventional ultrasound because there is a large difference in acoustic impedance between ice and water.

Figure 8 shows the first ever ultrasound image of a frozen lesion in the liver. The ultrasound transducer was placed on the outer surface of the liver (*white arrow*). The frozen region appears as a hemispherical dark area with a hyperecoic rim (*dark arrows*). Ice essentially reflects all the acoustic energy and therefore the entire area

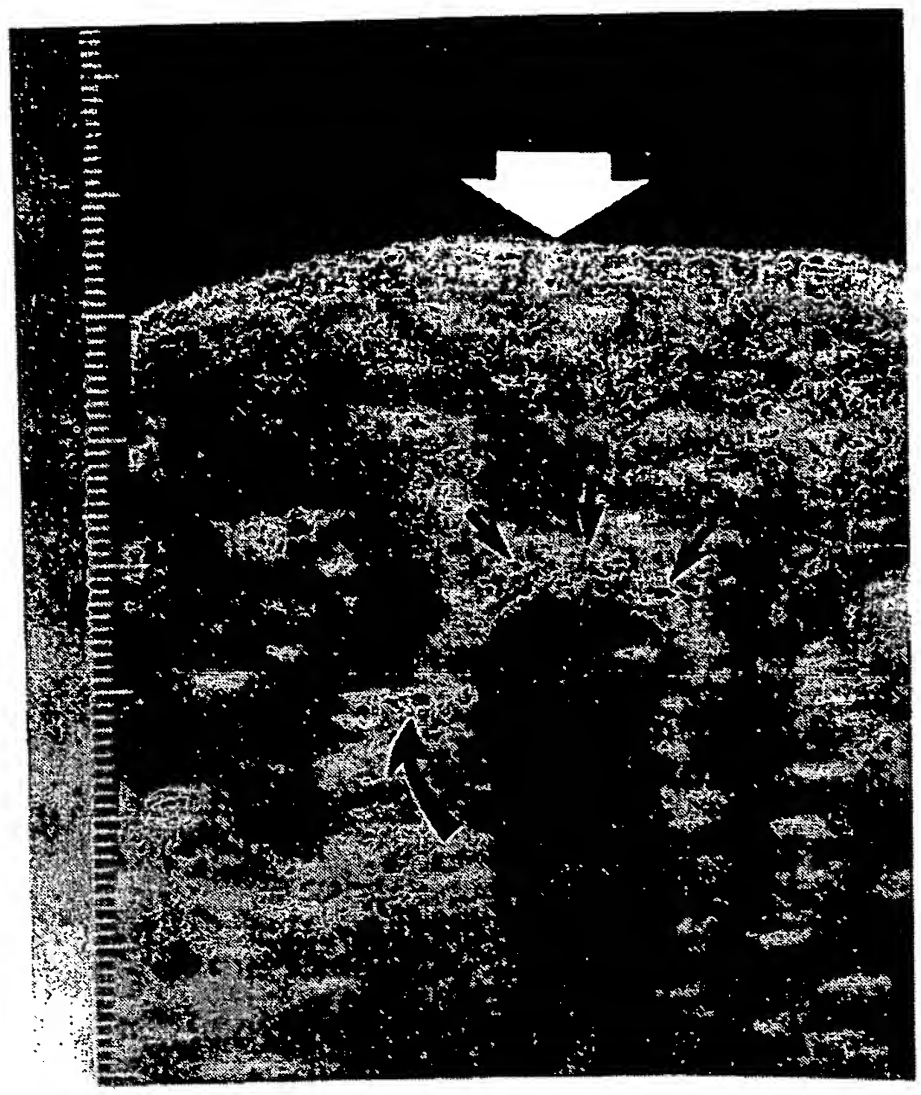


Figure 8 A typical ultrasound image of a frozen lesion in the liver. The *white arrow* indicates the outer surface of the liver where the ultrasound transducer was placed. The *black arrows* show the margin of the hemispherical frozen lesion as seen with conventional ultrasound.

behind the freezing interface is dark. This illustrates the problems with ultrasound. Because no acoustic energy can propagate through the frozen region, ultrasound can only image the freezing interface that faces the transducer. The opposite side of the frozen lesion is in the shade and cannot be imaged. Another problem is the inherent limitations of ultrasound. Because the acoustic wave is attenuated as it travels through the tissue, there is a limit to the depth of penetration and imaging.

Following the first report on the use of ultrasound in cryosurgery (92) a series of studies was published to characterize the procedure (93–96). The ability to image the frozen region deep in the body has led to the need for new cryosurgical instrumentation. The ability to view complex shapes led to the need for several cryosurgical probes that could be used simultaneously to achieve a desired ice shape. This multiprobe method had been reported in cryosurgery earlier. However, previous surgeons used several individual probes with their own liquid nitrogen

supply (97). Onik and Rubinsky and colleagues developed single unit multiple probe cryosurgical systems, which together with imaging, became the basis for the practice of cryosurgery during the 1990s (98). The first clinical reports on the use of cryosurgery to treat liver and prostate cancer were published soon after imaging-monitored cryosurgery and multiple cryosurgical probe systems were developed (99, 100). A comprehensive review of ultrasound imaging of cryosurgery, together with clinical applications of the technique can be found in 55. Currently, ultrasound-monitored cryosurgery is a clinically accepted technique for treatment of liver and prostate cancer, with close to two hundred sites and over ten thousand patients treated with this technique in the United States alone.

It appears that every known whole-body imaging technique can also be used to image the process of freezing during cryosurgery. Every imaging technique can potentially be used because every technique analyzes a certain tissue property, and all the properties of frozen tissue are different from those of unfrozen tissue. Magnetic resonance imaging (MRI) produces an image of the human body by applying an alternating magnetic field. The protons in water relax following the application of the magnetic field and the time of relaxation can be expressed in terms of two relaxation times T1 and T2. MRI essentially produces an image of proton density, which closely relates to tissue structure. The image is three-dimensional. Isoda was the first to demonstrate that MRI can be used to image freezing (101). Because the protons in ice have an entirely different relaxation time from those in water, the frozen region appears signal free in conventional MRI. In a subsequent study our group has shown that almost every MRI imaging technique can be used to image freezing, including fast and ultra-fast methods such as fast low-flip-angle, echo planar and gradient recalled echo (64, 102, 103). Using a MRI-compatible cryosurgical probe we have shown that T1-weighted imaging sequences can provide rapid images of the process of freezing. Figure 9 illustrates typical T1-weighted images obtained during brain cryosurgery. T2-weighted images are slower but produce a better contrast and therefore can be used to track postoperative events, such as local edema. Using contrast agents such as gadolinium, we have shown that MRI can detect the region in which blood flow is occluded after the frozen lesion thaws. With a newly designed cryosurgical probe that incorporates a MRI coil we produced better signal-to-noise ratio and successfully imaged freezing with a resolution of 40 microns (104). Because MRI produces a precise three-dimensional image of the freezing interface, it can be used to calculate the temperature distribution in the frozen region (105), and to provide real-time feedback for controlling cryosurgery (106). MRI is advantageous over ultrasound because it can produce a real-time three-dimensional image of the frozen lesion, without acoustic shadowing. However, it is much more expensive than ultrasound and it requires special surgical tools and a special environment. Nevertheless, because of the quality of the image, MRI-monitored cryosurgery is currently being tested in several clinical centers.

Magnetic resonance imaging provides an alternative to ultrasound that overcomes shadowing and generates a three-dimensional image. However, the high cost and unfriendly surgical environment of MRI may make this procedure too

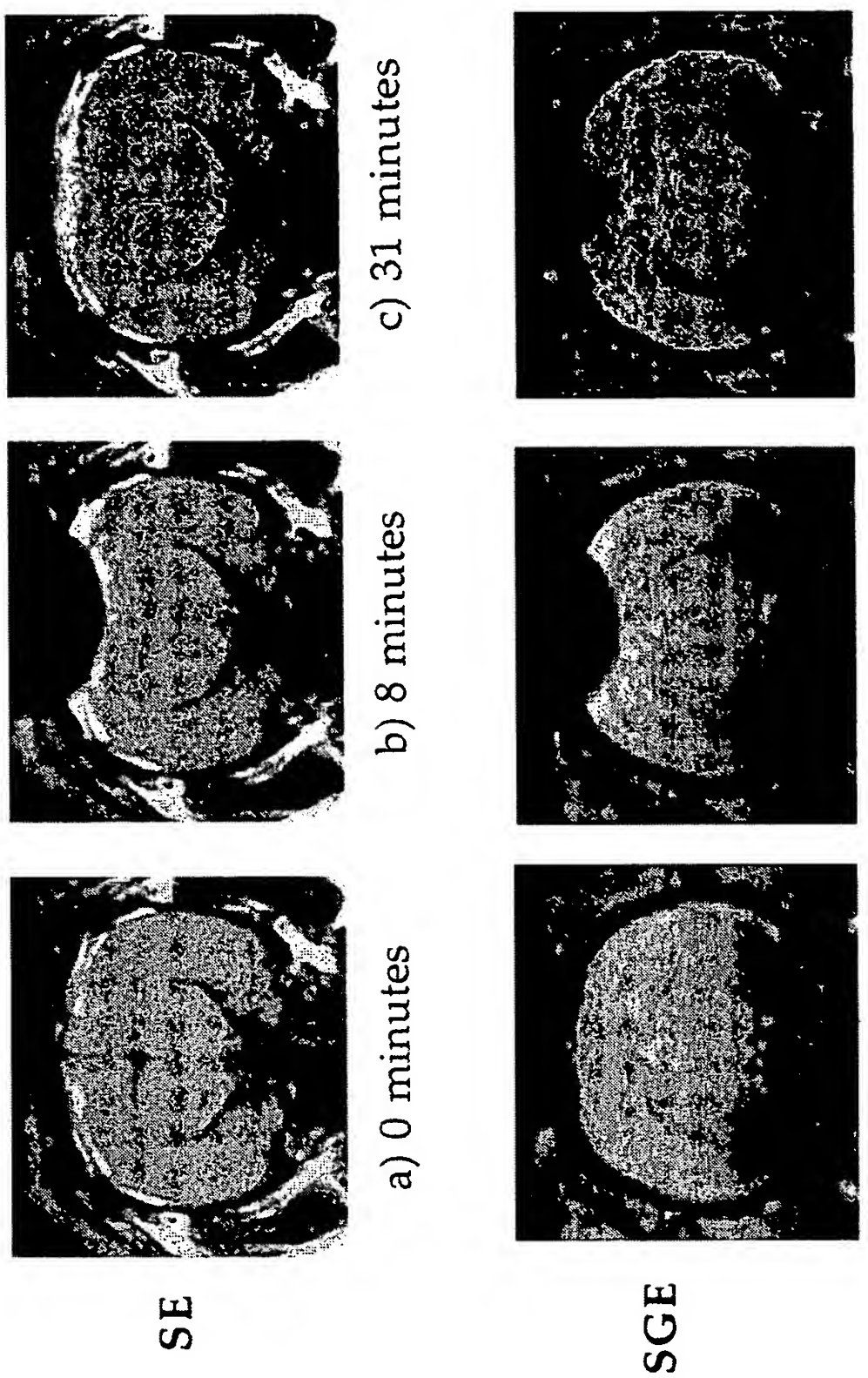


Figure 9 T1-weighted SE and SGE images before (*a*), during (*b*), and after (*c*) cryosurgery in the rabbit brain. The cryosurgery lesion is seen as a hemispherical region without signal at the top of *b*. T1-weighted SE images (TE = 28 ms, TR = 400 ms) were acquired in 2.6 min. SGE images (TE = 14 ms, TR = 50 ms) were acquired at a faster rate of 15 s/slice.

expensive to use, except at major clinical centers. Therefore, we began to search for alternative imaging techniques that could overcome the problems of ultrasound and be less expensive than MRI. One of these techniques is optical monitoring. Methods for applying optical spectroscopy and optical imaging to opaque, scattering media had already been developed (107). There are essentially two methods for optical imaging: One uses the time of flight of a proton through the tissue, and the other employs the scattering characteristics of the tissue. In both methods light is emitted on one surface of the tissue and detected on another. Tomography is then used to reconstruct the image from the optical properties of the tissue. To the eye, tissues appear to change during freezing. Therefore, we suspected that sufficient optical contrast should exist for monitoring the freezing process. In a series of papers we have demonstrated that optical imaging can indeed monitor the location of the freezing interface (108, 109).

Electrical impedance tomography (EIT) is another new technique that may provide an inexpensive and flexible supplement to existing cryosurgical monitoring techniques. Injecting small sinusoidal electrical currents into the body and measuring the resulting voltages through an electrode array produces a typical EIT image. An impedance image of the tissue is than produced from the voltage data using a reconstruction algorithm. Local impedancemetry techniques have already shown that tissue impedance changes upon freezing. Therefore, we assumed that EIT could also be used to image freezing. Analytical studies have shown that EIT can indeed detect freezing (110). Both optical imaging and EIT are techniques that are worth pursuing because of their low cost.

In summary, imaging modalities have provided physicians with the ability to monitor the process of freezing during cryosurgery. Imaging will most likely remain of importance to the field of cryosurgery as it develops. It is to be anticipated that new and better imaging modalities for cryosurgery will continue to develop as the fields of both cryosurgery and imaging mature.

SUMMARY

Cryosurgery is an important minimally invasive surgical technique. It can be applied to any procedure in which scalpels are used to remove undesirable tissues. Currently, cryosurgery is being used in many medical field, such as dermatology, gynecology, urology, neurology, pulmonary medicine, cardiology, oncology, and many others. Cryosurgery is also used in veterinary medicine. Imaging-monitored cryosurgery has revived the field, and numerous new applications are continuously emerging. With new applications came the need for better cryosurgical probes. New cryosurgical systems using supercooled liquid nitrogen, Joule-Thomson refrigeration with gas mixtures, closed cycle Stirling refrigeration, and heat pipe cooling have all been developed in recent years. I anticipate that cryosurgery will become a standard technique in the minimally invasive surgeon armamentarium. To improve cryosurgery further, there is the need to develop a better fundamental

understanding of the mechanisms of tissue damage during cryosurgery, to develop improved imaging techniques, new and improved cryosurgical device technology, and mathematical cryosurgery optimization techniques.

LITERATURE CITED

1. Arkharov A, Marfenina I, Mikulin Y. 1980. *Theory and Design of Cryogenic Systems.* Moscow: Mir. 430 pp.
2. Arnott J. 1850. The remedial efficacy of a low or anaesthetic temperature. *Lancet* 2: 257
3. Arnott J. 1851. *On the Treatment of Cancer by the Regulated Application of an Anesthetic Temperature.* 32 pp. London: Churchill
4. Cooke T. 1865. *On Cancer: Its Allies and Counterfeits.* London: Green. 116 pp.
5. Openchowski PH. 1883. Sur l'action localisee du froid applique a la surface de la region corticale du cerveau. Compte rend. *Soc. Biol.* 5:38–43
6. White AC. 1899. Liquid air: its application in medicine and surgery. *Med. Rec.* 56: 109–12
7. White AC. 1901. Possibilities of liquid air to the physican. *JAMA* 198:426–28
8. Whitehouse H. 1907. Liquid air in dermatology; its indications and limitations. *JAMA* 49:371–77
9. Bowen JT, Towle HP. 1907. Liquid air in dermatology. *Boston Med. Surg. J.* 157: 561–64
10. Gage AA. 1995. History. In *Cryosurgery: Mechanisms and Applications,* ed. L Lucas. Paris: Int. Inst. Refrig. 140 pp.
11. Pusey W. 1907. The use of carbon dioxide snow in the treatment of nevi and other lesions of the skin. *JAMA* 49:1534–36
12. Lortat-Jacobs L, Solente G. 1930. *La Cryotherapie.* Paris: Masson et Cie
13. Irvine H, Turnacliff D. 1929. Liquid oxygen in dermatology. *Arch. Dermatol. Syphilol.* 19:270–80
14. Hall FE. 1942. The use of quick freezing methods in gynecology practice. *Am. J. Obstet. Gynecol.* 43:105–11
15. Allington H. 1950. Liquid nitrogen in the treatment of skin diseases. *Calif. Med.* 72: 153–55
16. Fay T. 1959. Early experiences with local and generalized refrigeration of the human brain. *J. Neurosurg.* 16:239–59
17. Haas G, Taylor CA. 1948. Quantitative hypothermal method for production of local injury of tissue. *Arch. Pathol.* 45:563–80
18. Clasen R, Brown D, Leavitt S, Haas G. 1953. The production by liquid nitrogen of acute closed cerebral lesions. *Surg. Gynecol. Obstet.* 96:605–16
19. Rowbotham G, Haigh A, Leslie W. 1959. Cooling cannula for use in the treatment of cerebral neoplasms. *Lancet* 1:12–15
20. Tytus JS, Ries L. 1959. Further observations on rapid freezing and its possible application to neurosurgical techniques. *Bull. Mason Clin.* 15:51–61
21. Cooper I, Lee A. 1961. Cryostatic congelation: a system for producing a limited controlled region of cooling or freezing of biological tissue. *J. Nerv. Ment. Dis.* 133: 259–63
22. Gage A. 1986. In memoriam, Irving S. Cooper, MD. 1922–1985. *Cryobiology* 23: 287–89
23. Cooper I. 1963. Cryogenic surgery. *N. Engl. J. Med.* 743–49
24. Cooper IS. 1965. Cryogenic surgery for cancer. *Fed. Proc.* 24:743–49
25. Cahan W. 1964. Cryosurgery of the uterus: description of techniques and potential applications. *Am. J. Obstet. Gynecol.* 88: 410–14
26. Rand RW, Dashe AM, Paglia DE, Conway

LW, Solomon DH. 1964. Stereotactic cryohypophysectomy. *JAMA* 189:255–59
27. Gonder M, Soanes W, Smith V. 1964. Experimental prostate cryosurgery. *Invest. Urol.* 1:610–19
28. Marcove R, Miller T. 1969. The treatment of primary and metastatic bone tumors by cryosurgery. *Surg. Clin. N. Am.* 49:421–30
29. Torre D. 1970. Cutaneous cryosurgery. *NY State J. Med.* 70:2551–54
30. Zacarian S, Adham M. 1966. Cryotherapy of cutaneous malignancies. *Cryobiology* 2:212–18
31. Gage AA, Koepf D, Wehrle D, Emmings FG. 1965. Cryotherapy for cancer of the lip and oral cavity. *Cancer* 18:1646–51
32. Gage AA. 1968. Cryotherapy for inoperable rectal cancer. *Dis. Colon Rectum* 11: 36–44
33. Rinfret AP. 1966. Vacuum and cryogenics in biomedicine. *Ind. Res.* 3:75
34. Barron RF. 1968. Heat transfer problems in cryosurgery. *J. Cryosurgery* 1:316–25
35. Rand R, Rinfret A, von Leden H, eds. 1968. *Cryosurgery.* Springfield, IL: Thomas
36. von Leden H, Cahan W, eds. 1971. *Cryogenics in Surgery.* Flushing, NY: Med. Exam.
37. Cooper IS. 1964. Cryobiology as viewed by the surgeon. *Cryobiology* 1:44–54
38. Cahan W. 1965. Cryosurgery of malignant and benign tumors. *Fed. Proc.* 24:S241–48
39. Gill W, Frazer J, Carter D. 1968. Repeated freeze-thaw cycles in cryosurgery. *Nature* 219:410–13
40. Quinn PJ. 1985. A lipid phase separation model of low temperature damage to biological membranes. *Cryobiology* 22:128–47
41. McGrath JJ. 1988. Membrane transport properties. In *Low Temperature Biotechnology: Emerging Applications and Engineering Contributions,* ed. JJ McGrath, KR Diller, pp. 273–321 New York: ASME. 380 pp.
42. Wolfe J, Bryant G. 1999. Drying and/or vitrfication of membrane-solute-water systems. *Cryobiology* 39:103–30
43. Mazur P. 1970. Cryobiology: the freezing of biological systems. *Science* 68:939–49
44. Tatsutani K, Rubinsky B, Onik G, Dahiya R. 1996. Effect of thermal variables on frozen human primary prostatic adenocarcinoma cells. *Urology* 48:441–47
45. Ishiguro H, Rubinsky B. 1994. Mechanical interactions between ice crystals and red blood cells during directional solidification. *Cryobiology* 31:483–500
46. Kurtz W, Fisher DJ. 1984. *Fundamentals of Solidification.* Adermannsdorf, Switz.: Trans Tech SA
47. Lovelock JE. 1953. The haemolysis of human red blood cells by freezing and thawing. *Biochem. Biophys. Acta* 10:414–26
48. Tatsutani K, Rubinsky B. 1997. A technique to study the effects of cellular dehydration. *Cryobiology* 35(4):377
49. Nei T. 1967. Mechanism of hemolysis by freezing at near zero temperatures. II. Investigation of factors affecting hemolysis by freezing. *Cryobiology* 4:303–8
50. Mazur P, Rall WF, Rogopoulos N. 1981. Relative contributions of the fraction of unfrozen water and of salt concentration to the survival of slowly frozen human erythrocytes. *Biophys. J.* 36:653–65
51. Takamatsu H, Rubinsky B. 1999. Viability of deformed cells. *Cryobiology.* 39:243–51
52. Diller KR, Cravalho EG. 1970. A cryomicroscope for the study of freezing and thawing process in biological cells. *Cryobiology* 7:191–99
53. Toner M, Cravalho EG, Karel M. 1990. Thermodynamics and kinetics of intracellular ice formation during freezing of biological cells. *J. Appl. Phys.* 69(3):1582–93
54. Rubinsky B, Lee CY, Bastacky J, Onik G. 1990. The process of freezing and the mechanism of damage during hepatic cryosurgery. *Cryobiology* 27:85–97
55. Onik G, Rubinsky B, Watson G, Ablin

RJ. 1994. *Percutaneous Prostate Cryoablation.* St. Louis: Quality Med. 172 pp.
56. Hong JS, Rubinsky B. 1994. Patterns of ice formation in normal and malignant breast tissue. *Cryobiology* 31:109–20
57. Bischof J, Hunt CJ, Rubinsky B, Burgess A, Pegg DE. 1990. Effects of cooling rate and glycerol concentration on the structure of the frozen kidney: assessment by cryoscanning electron microscopy. *Cryobiology* 27:301–10
58. Rubinsky B, Pegg DE. 1988. A mathematical model for the freezing process in biological tissue. *Proc. R. Soc. London Ser. B* 234:343–58
59. Whitaker D. 1978. Electron microscopy of the ice crystals formed during cryosurgery: relationship to duration of freeze. *Cryobiology* 15:603–7
60. Merryman HT, ed. 1966. *Cryobiology.* New York: Academic. 966 pp.
61. Gage AA, Baust J. 1998. Mechanisms of tissue injury in cryosurgery. *Cryobiology* 37(3):171–86
62. Rubinsky B. 1982. Thermal stresses during solidification processes. *Trans. ASME: J. Heat Transf.* 104:196–99
63. Najimi S, Rubinsky B. 1997. Non-invasive detection of thermal stress fractures in frozen biological materials. *Cryo-Lett.* 18: 209–16
64. Gilbert JC, Rubinsky B, Roos MS, Wong ST, Brennan KM. 1993. MRI-monitored cryosurgery in the rabbit brain. *Magn. Reson. Imaging* 11:1155–64
65. Whittaker DK. 1984. Mechanisms of tissue destruction following cryosurgery. *Ann. Rev. Cool. Surg. Eng.* 66:313–18
66. Hollister WR, Mathew AJ, Baust JG, Van Buskirk RG. 1998. The effects of freezing on cell viability and mechanisms of cell death in an in vitro human prostate cancer cell line. *Mol. Urol.* 2:13–18
67. Ablin RJ, Soanes WA, Gonder MJ. 1971. Propsects for cryo-immunotherapy in cases of metastasizing carcinoma of the prostate. *Cryobiology* 8:271–79
68. Tanaka S. 1982. Immunological aspects of cryosurgery in general surgery. *Cryobiology* 19:119–28
69. Coger R, Rubinsky B, Fletcher G. 1994. Microscopic pattern of ice crystal growth in the presence of thermal hysteresis proteins. *Trans. ASME: J. Offshore Mech. Arctic Eng.* 116:173–79
70. Rubinsky B, DeVries AL. 1989. Effects of ice crystal habit on the viability of glycerol protected red blood cells. *Cryobiology* 26:580
71. Larese A, Acker J, Muldrew K, Yang HY, McGann L. 1996. Antifreeze proteins induce intracellular nucleation. *Cryo-Lett.* 17:175–82
72. Koushafar H, Rubinsky B. 1997. Effect of antifreeze proteins on frozen primary prostatic adenocarcinoma cells. *Urology* 49: 421–25
73. Koushafar H, Pham LD, Lee C, Rubinsky B. 1997. Chemical adjuvant cryosurgery with antifreeze proteins. *Cryobiology* 35: 324
74. Pham LD, Rubinsky B. 1998. Breast tissue cryosurgery with antifreeze proteins. In *Advances in Heat and Mass Transfer in Biotechnology,* pp. 171–75. New York: ASME Press
75. Pham LD, Dahiya R, Rubinsky B. 1999. An in vivo study of antifreeze protein adjuvant cryosurgery. *Cryobiology* 38(2):169–75
76. Cooper TE, Trezek GJ. 1971. Rate of lesion growth around spherical and cylindrical cryoprobes. *Cryobiology* 7:183–90
77. Cooper TE, Trezek GJ. 1972. On the freezing of tissue. *Trans. ASME: J. Heat Transf.* 94:251–53
78. Comini G, del Guidice S. 1976. Thermal aspects of cryosurgery. *Trans. ASME: J. Heat Transf.* 98:543–49
79. Rubinsky B, Shitzer A. 1976. Analysis of a Stefan-like problem in a biological tissue around a cryosurgical probe. *Trans. ASME: J. Heat Transf.* 98:514–19
80. Rubinsky B, Shitzer A. 1978. Analytic solutions of the heat equation involving a

moving boundary with application to the change of phase problem (the inverse Stefan problem). *Trans. ASME: J. Heat Transf.* 100:300–4
81. Keanini RG, Rubinsky B. 1992. Optimization of multiprobe cryosurgery. *Trans. ASME: J. Heat Transf.* 114:796–801
82. Rabin Y, Shitzer A. 1997. Combined solution of the inverse Stefan problem for successive freezing/thawing in non-ideal biological tissue. *Trans. ASME: J. Biomed. Eng.* 119:146–52
83. Rabin Y, Shitzer A. 1998. Numerical solution of the multidimensional freezing problem during cryosurgery. *Trans. ASME: J. Biomech. Eng.* 120:32–37
84. Pappenfmort RB. 1981. Semicircular thermocouple needle depth gauge for cryoprocedure. *Cutis* 27:605–8
85. Gage AA, Augustynowicz S, Montes M, Caruana JA, Whalen D. 1985. Tissue impedance and temperature measurements in relation to necrosis in experimental cryosurgury. *Cryobiology* 22:282–88
86. Revoire ML, Voiglio EJ, Kaemmerlen P. 1996. Hepatic cryosurgery precision: evaluation of ultrasonography, thermometry and impedancemetry in a pig model. *J. Surg. Oncol.* 61:242–48
87. Le Pivert PJ, Binder P, Ougier T. 1977. Measurement of intratissue bioelectrical low frequency impedance: a new method to predict per-operatively the destructive effect of cryosurgery. *Cryobiology* 14:245–50
88. Savic M, Zacarian SA. 1977. A new impedance-based method for controlled cryosurgery of malignant tumors. *J. Dermatol. Surg. Oncol.* 3(6):592–93
89. Gage AA. 1979. Correlation of electrical impedance and temperature in tissue during freezing. *Cryobiology* 16:56–62
90. Zacarian SA. 1980. How accurate is temperature monitoring in cryosurgery and is there an alternative? *J. Dermatol. Surg. Oncol.* 6:627–32
91. Wels PNT, ed. 1982. *Scientific Basis for Medical Imaging*. Edinborough/New York: Churchill Livingstone
92. Onik G, Cooper C, Goldenberg HI, Moss AA, Rubinsky B, Christianson M. 1984. Ultrasonic characteristics of frozen liver. *Cryobiology* 21:321–28
93. Onik G, Gilbert J, Haddick WK, Filly RA, Collen PW, Rubinsky B. 1985. Sonographic monitoring of hepatic cryosurgery in an experimental animal model. *Am. J. Roentgenol.* 144:1043–47
94. Gilbert JC, Onik GM, Hoddick WK, Rubinsky B. 1985. Real time ultrasonic monitoring of hepatic cryosurgery. *Cryobiology* 22:319–30
95. Lam CM, Shimi SM, Cuschieri A. 1985. Ultrasonic characterization of hepatic cryolesions. *Arch. Surg.* 130:1068–72
96. Rubinsky B. 1986. Cryosurgery imaging with ultrasound. *Mech. Eng.* 108:48–51
97. LLoyd-Williams K. 1975. In *Current Surgical Practice*, ed. J Hadfield, M Hobsley, pp. 243–67. London: Edward Arnold
98. Rubinsky B, Onik G, Finkelstein JJ, Neu D, Jones S. 1994. U.S. Patent No. 5334181
99. Onik G, Rubinsky B, Zemel R, Weaver L, Kane R, et al. 1991. Ultrasound-guided hepatic cryosurgery in the treatment of metastatic colon carcinoma. Preliminary results. *Cancer* 67:901–7
100. Onik GM, Cohen JK, Reyes GD, Rubinsky B, Chang ZH, Baust JG. 1993. Transrectal ultrasound-guided percutaneous radical cryosurgical ablation of the prostate. *Cancer* 72:1291–99
101. Isoda H. 1989. Sequential MRI and CT in monitoring cryosurgery—an experimental study in rats. *Nippon Acta Radiol.* 49: 1499–1508 (in Japanese)
102. Rubinsky B, Gilbert JC, Onik GM, Roos HS, Wong STS, Brennan KM. 1993. Monitoring cryosurgery in the brain and in the prostate with proton NMR. *Cryobiology* 30:191–99
103. Gilbert J, Onik G, Rubinsky B. 1998. MRI-guided tissue ablation using cryosurgery. In *Interventional MR Techniques and*

Clinical Experience, ed. F Jolesz, I Young, pp. 159–68. London: Dunitz
104. Pease GR, Wong STS, Roos MS, Rubinsky B. 1995. An integrated probe for magnetic resonance imaging monitored skin cryosurgery. *J. Biomech. Eng.* 117:59–63
105. Hong JS, Wong S, Pease G, Rubinsky B. 1994. MR imaging assisted temperature calculations during cryosurgery. *Magn. Res. Imaging* 12:1021–31
106. Pease GR, Wong ST, Roos MS, Rubinsky B. 1995. MR image-guided control of cryosurgery. *J. Magn. Res. Imaging* 5: 753–60
107. Benaron DA, Cheong W-F, Stevenson DK. 1997. Tissue optics. *Science* 276: 2002–3
108. Otten DM, Rubinsky B, Cheong WF, Benaron DA. 1998. Ice-front propagation monitoring in tissue by the use of visible-light spectroscopy. *Appl. Optics* 37:6006–10
109. Otten DM, Rubinsky B. 1998. Ice front propagation monitoring in tissue using visible light transmission: behaviour prediction using photon diffusion model. In *HTD*, 362:191–96. ASME Press
110. Radai MM, Abboud S, Rubinsky B. 1999. Evaluation of the impedance technique for cryosurgery in a theoretical model of the head. *Cryobiology* 38:51–60
111. Tatsutani KN. 1998. *The mechanism of freezing damage in prostate cryosurgery.* Ph.D. thesis. Univ. Calif., Berkeley. 145 pp.